Large Spinose Microfossils in Ediacaran Rocks as Resting Stages of Early Animals

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Large (>100 μm), profusely ornamented microfossils comprise a distinctive paleontological component of sedimentary rocks deposited during the Ediacaran Period (635–542 million years ago). Smaller spinose fossils in Paleozoic rocks have commonly been interpreted as algal cysts or phycomata, but the Ediacaran populations differ from modern algal analogs in size, shape, ultrastructure, and internal contents. In contrast, cysts formed during the diapause egg-resting stages of many metazoans share features of size, ornamentation, and internal contents with large ornamented Ediacaran microfossils (LOEMs). Moreover, transmission electron microscopic observations of animal-resting cysts reveal a 3-layer wall ultrastructure comparable to that of LOEM taxa. Interpretation of these distinctive Ediacaran microfossils as resting stages in early metazoan life cycles offers additional perspectives on their functional morphology and stratigraphic distribution. Based on comparisons with modern marine invertebrates, the recalcitrant life stage represented by LOEMs is interpreted as an evolutionary response to prolonged episodes of bottom water anoxia in Ediacaran shelf and platform environments. As predicted by this hypothesis, the later Ediacaran disappearance of LOEM taxa coincides with geochemical evidence for a marked decline in the extent of oxygen-depleted waters impinging on continental shelves and platforms. Thus, the form, diversity, and stratigraphic range of LOEMs illuminate life cycle evolution in early animals as influenced by the evolving redox state of the oceans.

acritarchs | Diapause egg cysts | origin of metazoans | paleoenvironment

Stratigraphically long-ranging prokaryotes and simple eukaryotic forms dominate a Proterozoic microfossil record nearly 2 billion years in duration. Viewed in this context, the Ediacaran radiation of large (generally >100 μm), often profusely-ornamented microfossils represents a major departure in the recorded history of life. Like the Ediacaran macrofossils of early animals (1), these distinctive microfossils first appeared in the wake of global glaciation and diversified over tens of millions of years. Unlike macroscopic Ediacaran fossils, however, large ornamented microfossils largely disappeared by ≈560 million years ago (Ma), if not earlier (2).

The Ediacaran microfossil radiation has been interpreted as an evolutionary response of protists to predation pressure from bilaterian animals, providing an indirect indication of early animal evolution (3). Yin et al. (4) (see also refs. 5 and 6), however, documented multicellular structures previously interpreted as early cleavage-stage animal embryos (7) inside large ornamented microfossils from *ca.* 580 ± 20 Ma rocks in China. This discovery suggests that the Ediacaran radiation of large ornamented microfossils may instead provide a direct record of early metazoans with a resting stage in their life cycle.

In this article, we provide further morphological and ultrastructural evidence that animal resting cysts are well represented in the Ediacaran microfossil record and explore the consequences of this conclusion in terms of terminal Proterozoic evolution and environmental history.

Large Ediacaran Microfossils: Systematic Interpretation

Large spheroidal microfossils, commonly with regularly arranged spines or other processes (large ornamented Ediacaran microfossils, or LOEMs) were first reported from Ediacaran cherts of the Doushantuo Formation, China (8) and have since been recorded globally (9). These microfossils have organic walls, and most are much larger than comparable Paleozoic fossils (100 to >500 μm in vesicle diameter, not including processes) (Fig. 1). Most also bear one to many spinose or branched processes distributed across vesicle surfaces. LOEMs are minor components of lower Ediacaran successions, but increase dramatically in both abundance and diversity at higher stratigraphic levels (2, 13). Where microfossils and carbon isotopic data are available for the same succession, LOEMs disappear within or just below an interval marked by a pronounced negative C-isotopic excursion tightly constrained by a 551 ± 0.7 Ma U-Pb date near its top but only loosely bracketed from below by *ca.* 600–621 Ma detrital zircons (Fig. 2 and refs. 27, 32, and 33). Uppermost Ediacaran strata do not contain large ornamented microfossils; instead they are dominated by simple spheroidal forms (34). Ornamented organic-walled microfossils radiated anew in the Early Cambrian, but Paleozoic forms are generally <50 μm in diameter (Fig. 1). Any hypothesis advanced to explain the biology and evolution of LOEM taxa must account for their distinctive features of morphology and stratigraphy.

Evaluation of Candidate Relationships. Variably-ornamented, organic-walled microfossils occur widely in Lower and Middle Paleozoic marine rocks. Called acritarchs, they are classified as problematica, but commonly interpreted as algae. More than a decade ago, however, van Waveren and Marcus (35) emphasized the morphological similarities between some of these fossils and diapause egg cysts produced by copepods and other animals.

Among extant phytoplankton groups, dinoflagellates and green algae include species that produce decay-resistant cell walls at some point in their life cycle. Resting stages with recalcitrant walls also occur in most major clades of animals, including the gemmules of sponges, cnidarian podocysts, and the egg and diapause cysts of diverse bilaterian metazoans (10, 36–38). Other protists are known to produce recalcitrant cysts, but to the best of our knowledge, none provide a close match for the Ediacaran fossils under consideration. Green algae, dinoflagellates, and animals, then, provide the principal actualistic comparisons to LOEM taxa. Observable characters that can be used to evaluate hypotheses of systematic relationship include size, shape, ultrastructure, internal contents, and, in principle, wall chemistry.

A cardinal feature of LOEMs is their size. More than 80% of described species have diameters >100 μm, and half exceed 200 μm (Fig. 1). This immediately casts doubt on dinoflagellate affinities, because most modern and fossil dinocysts are 30–80 μm in diameter; dinocysts >120 μm are rare, and examples >200 μm are unknown (39, 40). [Diffusion within cells and through the

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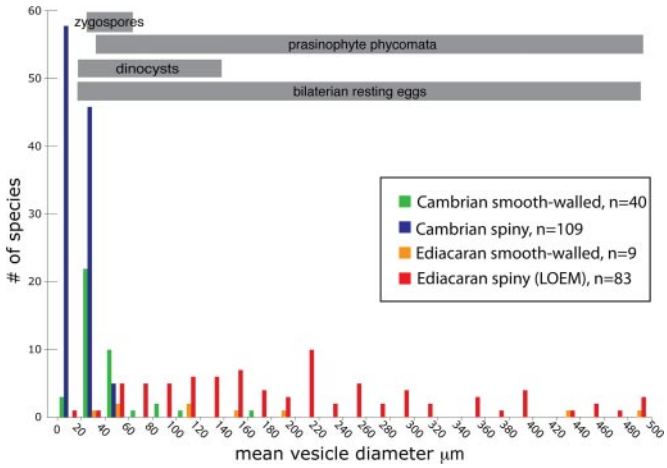

Fig. 1. Size frequency distributions of Ediacaran and Cambrian acritarch species, with modern analog ranges. Data are from refs. 7–26.

boundary layer limits cell size in extant phyoplankton that lack large vacuoles; very likely similar biophysical factors limited algal cell size in the past (41).] Moreover, at least some large Ediacaran microfossils preserve multicellular contents (4, 5, 11), an observation inconsistent with dinoflagellate life cycles (42). The interpretation of LOEM taxa as dinocysts also runs afoul of evidence from lipid biomarkers; diagnostically dinoflagellate steranes are rare in Ediacaran bitumens and do not become common until the Mesozoic Era, coincident with the appearance

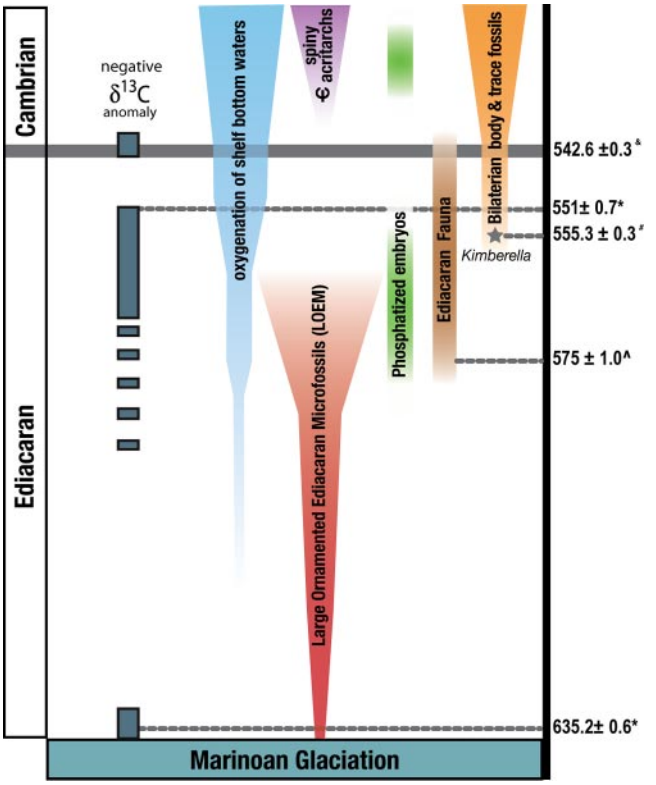

Fig. 2. Stratigraphic relationships of major events in Ediacaran oceans. Dates are indicated by: # (28), * (27), ^ (29), and & (30). Carbon isotope data are from refs. 2, 27, and 31. Geochemical data suggesting oxygenation of the oxygen miniumum zone are bracketed by the beginning and end of the so-called Shuram event, the large negative C isotopic anomaly within the Ediacaran Period (2, 76, 77, 81).

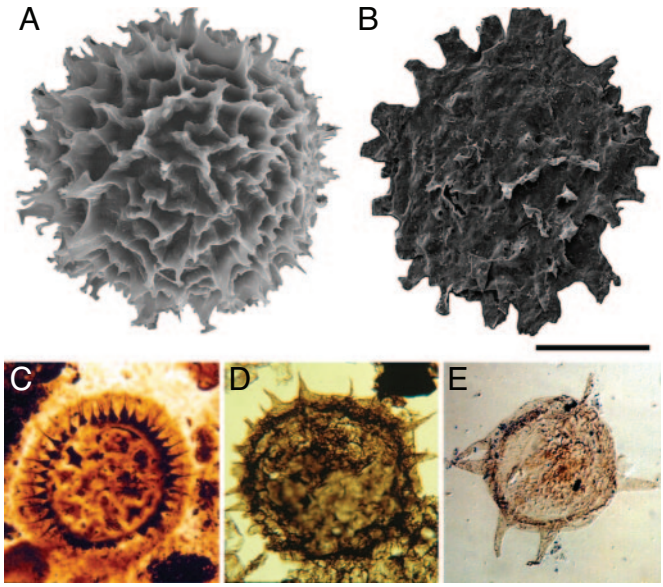

Fig. 3. Morphologies of LOEM taxa and a modern analog. (*A*) SEM of resting cyst, modern arthropod *Brachinella longirostris*. (*B*) *Alicesphaeridium* sp., Vychegda Formation, northern Russia. (*C*) LOEM microfossil, Doushantuo Formation, China. (*D*) LOEM microfossil, Officer Basin, Australia. (*E*) LOEM microfossil, Kursovsky Formation, Siberia. (Scale bar: 100 μm for *A* and *D*; 200 μm for *B* and *C*; 150 μm for *D* and *E*.)

of abundant, morphologically-diagnostic dinoflagellate microfossils (43, 44).

Prasinophytes, phytoflagellates that form a paraphyletic base to the green algal tree, include species that make reproductive structures called phycomata, which can be up to 500 μm in diameter (Fig. 1). Prasinophyte phycomata occur as microfossils in Phanerozoic shales, and Arouri et al. (45) have documented distinctively phycomate ultrastructure in a large spheroidal microfossil from Ediacaran strata in Australia. No known living or fossil prasinophyte, however, produces spinose structures comparable to those in Ediacaran rocks. In contrast, chlorophycean zygospores and the resting cysts of some other derived green algae may bear spinose or branched processes, but are much smaller than LOEMs (an order of magnitude in diameter; hence, 3 orders of magnitude by volume). Arouri et al. (46) identified aliphatic polymers called algaenans in the walls of some LOEMs, and on this basis they suggested green algal relationships; however, in the modern biota, algaenan production is largely limited to 2 specific clades of nonmarine green algae, and there is reason to believe that at least some of the algaenans in ancient marine rocks originated during diagenesis (47).

The resting stages of invertebrate eggs and embryos encompass the full range of sizes observed in LOEM taxa (Fig. 1) and display a comparable range of morphologies (Fig. 3). Indeed, among extant organisms, metazoans are the only group known to produce preservable structures that match LOEM taxa in both size and morphology. We do not argue that cysts of living animals provide a precise systematic guide to LOEMs; available evidence suggests that Ediacaran animals included stem group metazoans, eumetazoans, and bilaterians only broadly related to extant metazoans (7). We are more confident, however, in making the converse argument. If found as fossils, the diapause stages produced by living animals would be assigned to genera erected for the description of Ediacaran microfossils. Comparisons based solely on external morphology and size are incomplete, however, because of potential convergence. Thus, we turn to

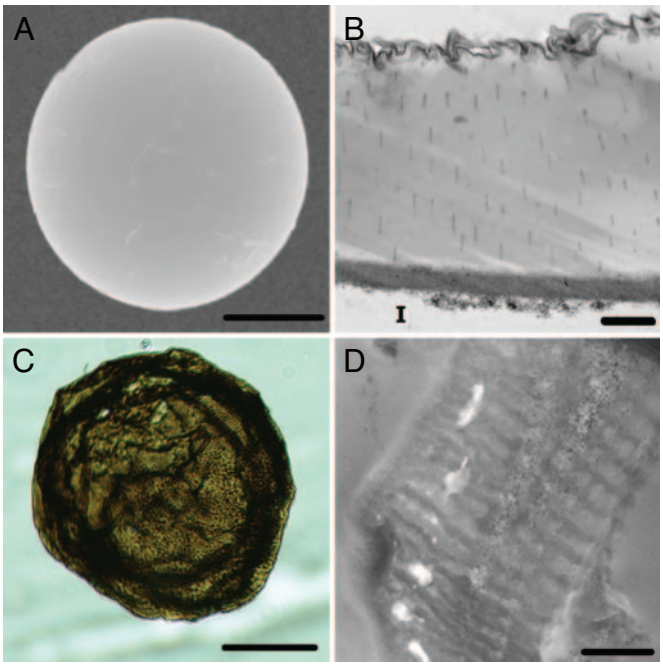

Fig. 4. Comparison of a modern algal analog and Ediacaran smooth-walled microfossil. (*A*) *Halosphaera* sp. phycoma from Puget Sound. (*B*) TEM of *Halosphaera* sp. phycoma. I indicates inside of phycoma. (*C*) Ediacaran *Leiosphaera* sp. (*D*) TEM Ediacaran *Leiosphaera* sp. (Scale bars: *A* and *C*, 50 μm; *B* and *D*, 500 nm.)

ultrastructure to evaluate further the systematic relationships between LOEM fossils and their extant analogs.

Insights from Ultrastructure. Transmission electron microscopy (TEM) provides a strong test of proposed systematic affinities. For example, the walls of some prasinophyte phycomata, including the genera *Pachysphaera*, *Halosphaera*, and *Cymbomonas*, have a distinctive ultrastructure marked by radially-oriented punctae (refs. 48–50 and Fig. 4 *A* and *B*) that, as noted above, has been recorded in large sphaeromorphic microfossils from Ediacaran rocks (45). Other Ediacaran sphaeromorphs, however, preserve ultrastructural features incompatible with a prasinophyte interpretation (Fig. 4 *C* and *D* and see also ref. 51), as do some sphaeromorphs from both older and younger rocks (52, 53).

TEM observations show that some LOEMs preserve a complex wall structure, with discrete layers of varying electron density (45, 54). We analyzed additional LOEM specimens, with similar results, seen clearly in *Gyalsosphaeridium* sp. from the Ediacaran Officer Basin of Australia (Fig. 5*A*). This specimen shows an electron-dense outer layer that extends outward to form processes (Fig. 5 *B* and *C*), a fibrous and electron tenuous middle layer, and a thin, electron-dense inner layer. Preserved as an organic walled structure in siliciclastic sediments, this specimen and others like it represent only the recalcitrant outer wall of a resting stage. The ultrastructure of *Gyalsosphaeridium* sp., however, is consistent with taphonomic pattern displayed by silicified LOEMs, which commonly show the diagenetic separation of an outer, process-bearing layer from the internal wall and sometimes preserve internal contents lost to decay in organic-walled forms (4–6, 11).

The ultrastructure of examined LOEM taxa clearly differentiates them from punctate phycomata, and, despite having multiple layers, it does not closely resemble the trilaminar ultrastructure of other green algal walls (55). How, though, does it compare with the resting cysts of animals? To address this question, we imaged diapause cysts of the brine shrimp *Branchinella longirostris* (Fig. 5*D*). Under TEM, the cyst wall exhibits a thin, electron-dense outer layer from which processes arise (Fig. 5*E Inset*), a thicker, more electron-tenuous and fibrous middle layer (Fig. 5*F*), and an inner layer, similar in electron density to the outer wall but thinner. Comparable ultrastructure has been documented for other arthropods (56, 57). Like its external morphology, then, the ultrastructure of *B. longirostris* compares closely with those of observed LOEM taxa (Fig. 3).

Systematic Conclusions. Based on available evidence from size, morphology, ultrastructure, and internal contents, the most compelling interpretation of LOEM taxa is that they are metazoan resting stages. We do not claim that all early animals had a resting stage in their life cycle; taphonomic experiments (58) indicate that cyst-forming animals should be differentially well-represented in the fossil record. Nor do we claim that all LOEMs must be metazoan cysts. Not all have close morphological counterparts among living animals, although the stratigraphic coherence of these fossils makes such a view tenable. Certainly, however, species assigned to such genera as *Alicesphaeridium*, *Appendipshaera*, *Cerionopora*, *Gyalosphaeridium*, *Ericiasphaera*, *Dicrospinosphaera*, *Sinosphaera*, *Tanarium*, *Tianzhushania*, *Taedigerasphaera*, and *Vidalia* have attributes of size, shape, and, where known, ultrastructure and internal contents consistent with interpretation as metazoan cysts. Although these are undoubtedly only a subset of early to mid-Ediacaran animal diversity, they form the most diverse record of early animals currently available.

Large spheroidal microfossils that are unornamented (e.g., Fig. 4 *C* and *D*) may also include animal cysts, although this interpretation must be evaluated on a case-by-case basis using TEM. We note that large organic walled microfossils with regularly-arranged processes on the vesicle surface are rare in pre-Ediacaran assemblages, with only a single instance documented from Mesoproterozoic shales (59). *Shuiyousphaeridium maeroreticulatum* exhibits regularly-arranged cylindrical processes that flare outward; its ultrastructure, however, is distinct from those of LOEMs (52).

Cyst formation has been documented in freshwater choanoflagellates (60), but these differ markedly from animal cysts in both size (a few microns) and shape (flask-shaped). Both molecular clocks (32, 33) and biomarker molecules indicate that sponges had evolved by the time the Marinoan glaciation ended (61); thus, LOEM diversification corresponds to the time of initial animal divergence and likely documents the diversification of stem and early crown group metazoans.

Implications for Ediacaran Evolution and Environmental Change

Peterson and Butterfield (3) interpreted Ediacaran microfossil morphologies as a defensive response to the evolution of cell-ingesting animal predators. Such an interpretation is plausible, but has at least 2 weaknesses. First, the spines-as-defense hypothesis is explicitly predicated on the assumption that protistan cells were not subject to predation before the evolution of eumetazoans. Many protists feed primarily on bacteria or small organic particles, but the ingestion of whole eukaryotic cells is also widespread. Indeed, testate amoebae, planktonic foraminifera, and the large ciliate *Stentor* all have been observed to capture and digest small animals and other protists (62–65). Thus, to the extent that spinose cysts are protective, they might well have been required as defense against protistan predators that were present in the oceans long before the Ediacaran Period (66). Spines and other ornamentation that effectively increase cell size may actually provide better protection against protistan predators than they do against invertebrates, especially if early

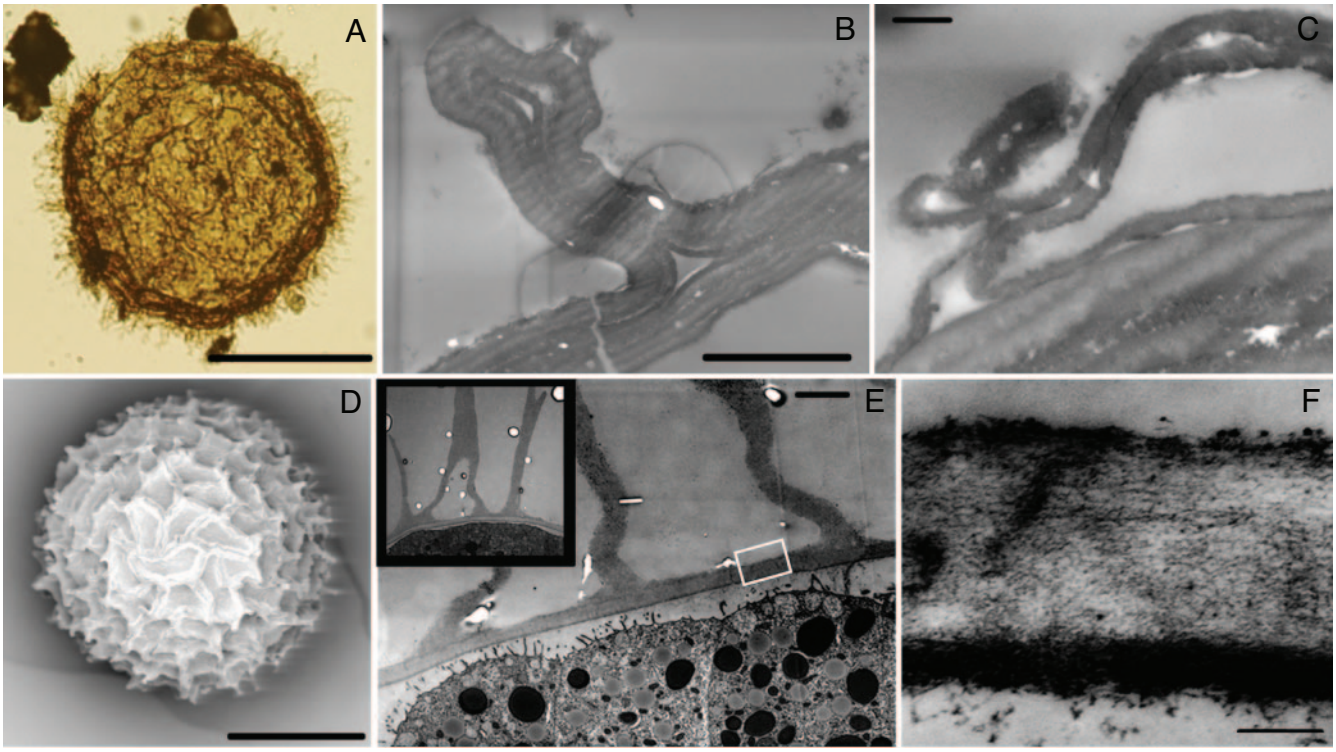

Fig. 5. Comparison of a LOEM fossil and a modern crustacean analog. (*A–C*), *Gyalosphaeridium* sp. (*A*) Light micrograph. (*B* and *C*) TEM. (*D–F*) *Branchinella longirostris.* (*D*) SEM. (*E*) TEM. (*Inset*) Hollow process. (*F*) TEM of outer wall. (Scale bars: *A* and *D*, 100 μm; *B* and *C*, 500 nm; *E*, 4 μm; *F*, 200 nm.)

animals were filter feeders that would have more easily entrapped ornamented forms. When offered dinoflagellates in feeding experiments, copepods choose unencysted prey over encysted prey, but do not distinguish among cysts with differing external morphologies (67). In many cases, encysted dinoflagellates and diatoms simply pass through the gut of predators unharmed (67, 68), which suggests that whereas encystment itself may be a useful strategy for avoiding predation, resource-costly spines are not necessarily so.

The second weakness of the spines-as-defense hypothesis is stratigraphic: LOEM taxa disappear at about the time when trace and body fossil evidence of bilaterian animals first appears (28, 69). That is, just when LOEMs should become most useful as defense against cell ingesting metazoans, they exit the record (Fig. 2).

If LOEM taxa are interpreted as metazoan, why might early animals have evolved the capacity to encyst? Along modern shorelines, many species produce encysted resting stages that accumulate in sediments (70). Resting stages that have been studied in detail have resistant multilayered walls, contain abundant lipids as metabolic reserves, and have highly suppressed metabolic rates (67). Although predation pressure can induce cyst formation in modern lakes (71), encystment in marine settings often occurs in response to physical rather than biological challenges. For example, observations of marine copepods show that anoxia can be a factor in the induction of a diapause stage, causing females to switch production from subitaneous (immediately hatching) to resting eggs (72, 73). Such resting stages can settle into marine sediments and remain for years or even decades before activating their ontogeny (71, 74). Experimental data show that hypoxia completely inhibits hatching in a number of animals; when returned to normoxic conditions after an interval of months to years, the same eggs hatch with high rates of viability (75–77). Modern studies, thus, show that deleterious environmental conditions are a powerful factor in inducing diapause in marine organisms. Additionally, taphonomic experiments on *Artemia* diapause cysts show that resting stages have high preservation potential (58). In summary, then, modern comparisons indicate the physical environment may have played a role in the appearance of LOEM taxa in Ediacaran oceans and emphasize the fossilization potential of such recalcitrant ontogenetic stages.

Considering these data, we suggest that some early animals had a protective resting stage in their life cycles to accommodate variable and potentially-lethal environmental conditions, including anoxia. A resting stage would be highly adaptive where the probability was high that broadcast eggs would land in an environment unfavorable for growth. Like the spines-as-defense hypothesis, our view is predicated on function, but a different and well-established function of cysts in modern environments. It is linked to animal evolution directly in terms of life cycle dynamics, as opposed to indirectly through ecology. Moreover, our hypothesis provides a direct link to environmental history of the oceans. Although the first appearance and radiation of LOEMs reflect, in this view, early animal diversification in unstable and commonly unfavorable environments, their later Ediacaran disappearance finds ready explanation in the increased oxygenation of the bottom waters that covered marine shelves and platforms. The loss of LOEM fossils coincides stratigraphically with geochemical data from C and S isotopes, Fe speciation chemistry, and Mo abundances in shales that collectively indicate the oxygenation of previously widespread anoxia in the oxygen minimum zones of the world's oceans (2, 78–80). Thus, the predictions this hypothesis makes about the stratigraphic relationship between LOEM fossils and geochemical records of Ediacaran environmental evolution are borne out by integrated geochemical and micropaleontolgical data.

In light of the above observations, it seems clear that early metazoans had to contend with water column redox conditions marked by pronounced spatial and temporal variation through all phases of their life cycles. The development of a protected egg

or embryo stage that could withstand adverse conditions would have enabled early animals to survive through protracted intervals of seafloor anoxia, only reactivating development when ambient waters or sediments transitioned to more favorable conditions. The time scales on which these transitions may have occurred can also account for the large size of LOEM taxa. In modern marine invertebrates, larger egg size enables organisms to survive in resting stages for longer periods of time, because larger size enables enhanced storage of the lipids required to maintain a highly suppressed but still active metabolism (81). Viewed physiologically, then, the size of LOEM taxa could reflect a need to maintain resting metabolism over potentially long periods of time.

Our hypothesis also has the potential to explain the highly ornamented nature of LOEM taxa. The majority of modern marine resting stages show sculpted or spiny coverings, in contrast to subitaneous eggs, which rarely have ornamentation (70). Thus, in modern groups, spines appear to play a role in survival in the marine sedimentary environment. One possible role could be that during episodic disturbance of the sediment cysts with spines will stay suspended above the sediment–water interface longer than settling sediments, allowing organisms to perceive changes in water column conditions that may induce excystment (70). In modern environments, spinose cyst morphologies commonly record a life history response to the physical environment, and by analogy or homology, LOEM taxa may record the same life history strategy in Ediacaran animals.

Conclusions

The recognition that some Ediacaran microfossils were metazoan prompts the question of whether animal cysts may lurk among the diverse acritarchs in Paleozoic rocks. It has been noted that Paleozoic acritarch diversity mirrors the diversification of marine invertebrates, a correspondence generally interpreted in terms of trophic interactions (82, 83). Without question, algal microfossils are present in Paleozoic acritarch assemblages, but only careful TEM imaging will establish to what extant Paleozoic acritarchs provide direct versus indirect indications of animal diversity, and whether the later Paleozoic collapse of acritarch diversity records extinction among primary producers, life-cycle alterations in response to a long-lasting state change in marine redox profiles, or a combination of the two.

In any event, multiple lines of evidence support the hypothesis that some large, ornamented, organic walled microfossils in Ediacaran sedimentary rocks record resting stages of early animals. This conclusion leads to the hypothesis that LOEM taxa are a life stage evolved by early animals in response to challenging environmental factors faced by organisms in Ediacaran seas. The disappearance of LOEMs coincides with geochemical evidence for widespread oxygenation of the seafloor, removing a major impetus for resting stage formation. In this view, true biological extinction may not have governed the Ediacaran microfossil record; the disappearance of LOEM taxa could reflect life-cycle evolution in early animals. The pattern of LOEM diversity observed in Ediacaran rocks may, thus, be a combination of true taxonomic changes and an evolutionary and physiological response to dramatic transitions in the biogeochemical conditions of the world's oceans.

Materials and Methods

All fossil samples used for ultrastructural analysis are from the Giles 1 core, Officer Basin, Australia, meter levels 430 and 427.6. Nine specimens, 6 acanthomorphic and 3 smooth-walled, were examined by TEM. Information on locality and stratigraphy can be found in ref. 84. Fossils were macerated directly from core samples at the Harvard University Botanical Museum according to the methods in ref. 85. *Halosphaera* phycomata were collected in January and Feburary, 2006, from northern Puget Sound, Washington and were fixed in 2% gluteraldehyde in sea water and transferred to distilled water through a series of washes with decreasing ratios of sea water/distilled water in 20% increments. Samples were then postfixed in 2% osmium tetroxide for 1 h at 4 °C, then washed with distilled water and stored at 4 °C. For TEM, both fossil and modern samples were dehydrated with ethanol in successive 20% increasing steps for 1 h at each step. Samples were embedded in a mixture of 50%–50% Epon epoxy and ethanol for 1 h, 30%–70% for 12–24 h, then 100% Epon for 1 h under vacuum. Samples were embedded in a thin film and hardened in a 60 °C oven for 12–24 h. Specimens were cut out and remounted on blank capsules for microtoming with a diamond knife and mounted on copper or Formvar-coated grids. Grids were stained by using uranyl acetate and lead citrate to improve contrast and examined with a JEOL 2100 TEM or a Zeiss SupraVP S-TEM.

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